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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF DOLUTEGRAVIR AND LAMIVUDINE IN PURE SUBSTANCES AND MARKETED PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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Abstract:

A reverse phase liquid chromatographic method for estimation of Dolutegravir and Lamivudine in bulk drugs and marketed pharmaceutical dosage form was developed and validated. The chromatographic conditions to achieve the highest performance parameters using Altima C_{18} (4.6×150mm, 5.0 µm) Column with guard filter were optimized. The separation was carried out using a mobile phase containing Methanol: TEA Buffer pH 4.5: Acetonitrile was taken in the ratio of 50: 25: 25% v/v/v pumped at a flow rate of 1.0 mL/min with detection at 225 nm. The method was shown to be linear in 5–25 µg/mL and 12.5–50 µg/mL concentration range (regression coefficients of 0.9993 and 0.9995) for Dolutegravir and Lamivudine respectively. The limit of detection (LOD) and limit of quantification (LOQ) was found to be 0.2µg/mL and 0.8µg/mL & 2.3µg/mL and 7.04µg/mL for Dolutegravir and Lamivudine respectively. The accuracy of the method was assessed by adding fixed amount of pre-analyzed sample to different standard solutions (50%, 100%, and 150% of the tested concentration) in triplicate. The percentage mean recoveries were found to 98%-102%. The method was found to be precise with %RSD value was found to be within the limits for intraday and interday precision study, respectively. The method specificity and robustness were also established. New and sensitive RP-HPLC method for estimation of Dolutegravir and Lamivudine has been developed, in respect to the reviewed analytical methods.

Key Words: *Dolutegravir and Lamivudine, RP-HPLC, Accuracy, Precision, Robustness.*

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INTRODUTION:

Dolutegravir is indicated in combination with other antiretroviral agents for the treatment of patients with HIV-1 infection that comply with the characteristics of being adults or children aged 12 years and older and present at least a weight of 40 kg.7 The FDA combination therapy approval of Dolutegravir and Rilpivirine is indicated for adults with HIV-1 infections whose virus is currently suppressed (< 50 copies/ml) on a stable regimen for at least six months, without history of treatment failure and no known substitutions associated to resistance to any of the two components of the therapy. The IUPAC name of Dolutegravir (3S, 7R)-N-[(2, 4-difluoro phenyl) methyl]-11-hydroxy-7-methyl-9, 12-dioxo-4-oxa-1, 8-diazatricyclo [8.4.0.03, 8] tetradeca-10,13-diene-13-carboxamide. The Chemical Structure of Dolutegravir is shown in follows

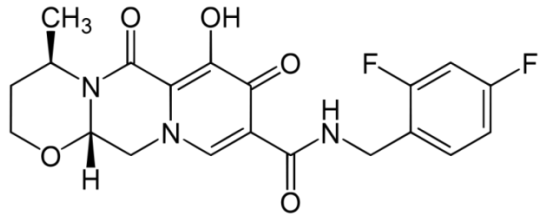

Fig-1: Chemical Structure of Dolutegravir

A reverse transcriptase inhibitor and Zalcitabine analog in which a sulfur atom replaces the 3' carbon of the pentose ring. It is used to treat Human Immunodeficiency Virus Type 1 (HIV-1) and hepatitis B (HBV). Lamivudine is a nucleoside analogue and reverse transcriptase inhibitor used in the therapy of human immunodeficiency virus (HIV) and hepatitis B virus (HBV) infection. Lamivudine (Epivir-HBV) is used to treat hepatitis B infection. Lamivudine is in a class of medications called nucleoside reverse transcriptase inhibitors (NRTIs). It works by decreasing the amount of HIV and hepatitis B in the blood. The IUPAC name of Lamivudine is 4-amino-1-[(2R, 5S)-2-(hydroxy methyl)-1, 3-oxathiolan-5-yl] pyrimidin-2-one. The Chemical Structure of Lamivudine is shown in following figure-1.

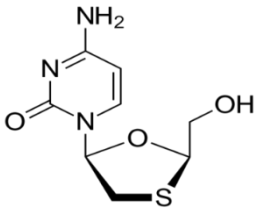

Fig-2: Chemical Structure of Lamivudine

Literature survey revealed a few methods reported for the simultaneous determination of Dolutegravir and Lamivudine in bulk drug as well as pharmaceutical preparation[33-36]. In this research, a new sensitive and rapid HPLC method was developed for the determination of Dolutegravir and Lamivudine in bulk and pharmaceutical dosage forms, and this method was validated according to ICH and FDA guidelines[20-21].

EXPERIMENTAL METHODS

Table-1: Instruments Used

S.No.	Instruments and Glasswares	Model
1	HPLC	WATERS Alliance 2695 separation module, software: Empower 2, 996 PDA detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital Ultra Sonicator	Labman

Table-2: Chemicals Used

S.No.	Chemical	Brand Names
1	Dolutegravir	Synpharma Research Lab, Hyderabad
2	Lamivudine	
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck

HPLC Method Development:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Dolutegravir and Lamivudine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.1ml of the above Dolutegravir and 0.375ml of the Lamivudine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines[20-21].

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water and Water: Acetonitrile and Methanol: TEA Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Methanol: TEA Buffer: ACN in proportion 50:25:25 v/v respectively[1].

Optimization of Column:

The method was performed with various columns like C18 column, Symmetry and Zodiac column. Altima C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow[2].

Preparation of Triethylamine (TEA) buffer (pH-4.5):

Dissolve 1.5ml of Triethyl amine in 250 ml HPLC water and adjust the p^H 4.5. Filter and sonicate the solution by Vaccum filtration and ultrasonication[3].

Preparation of Mobile Phase:

Accurately measured 400 ml (40%) of Methanol, 200 ml of Triethylamine buffer (20%) and 400 ml of Acetonitrile (40%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration[4].

Diluent Preparation:

The Mobile phase was used as the diluent.

Validation Parameters

System Suitability

Accurately weigh and transfer 10 mg of Dolutegravir and 10mg of Lamivudine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Dolutegravir and 0.375ml of the Lamivudine stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent[5].

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits[6].

Specificity Study of Drug:

Preparation of Standard Solution:

Accurately weigh and transfer 10mg of Dolutegravir and 10mg of Lamivudine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Dolutegravir and 0.375ml of the Lamivudine stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Sample Solution:

Take average weight of one Tablet and crush in a mortor by using pestle and weight 10 mg equivalent weight of Dolutegravir and Lamivudine sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.1ml of the above Dolutegravir and 0.375ml of the Lamivudine stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent[7].

Procedure:

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Preparation of Drug Solutions for Linearity:
Accurately weigh and transfer 10 mg of Dolutegravir and 10mg of Lamivudine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent[8-9]. (Stock solution)

Preparation of Level – I (5 ppm of Dolutegravir & 12.5ppm of Lamivudine):
Pipette out 0.05ml of Dolutegravir and 0.125ml of Lamivudine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (10 ppm of Dolutegravir& 25ppm of Lamivudine):
Pipette out 0.1ml of Dolutegravir and 0.25ml of Lamivudine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (15 ppm of Dolutegravir& 37.5ppm of Lamivudine):
Pipette out 0.15 ml of Dolutegravir and 0.375ml of Lamivudine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (20 ppm of Dolutegravir& 50ppm of Lamivudine):
Pipette out 0.2 ml of Dolutegravir and 0.5ml of Lamivudine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (25 ppm of Dolutegravir& 62.5ppm of Lamivudine):
Pipette out 0.25ml of Dolutegravir and 0.625ml of Lamivudine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure:
Inject each level into the chromatographic system and measure the peak area.
Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient[10-12].

Precision
Repeatability
Preparation of Dolutegravir and Lamivudine Product Solution for Precision:
Accurately weigh and transfer 10 mg of Dolutegravir and 10mg of Lamivudine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Dolutegravir and 0.375ml of the Lamivudine stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits[13].

Intermediate Precision:
To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:
Day 1:
The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits.
Day 2:
The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits[14].

Accuracy:
For preparation of 50% Standard stock solution:
Accurately weigh and transfer 10 mg of Dolutegravir and 10mg of Lamivudine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.075ml of the above Dolutegravir and 0.187ml of the Lamivudine stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 100% Standard stock solution:
Accurately weigh and transfer 10 mg of Dolutegravir and 10mg of Lamivudine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15ml of the above Dolutegravir and 0.375ml of the Lamivudine stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent[15].

For preparation of 150% Standard stock solution:
Accurately weigh and transfer 10 mg of Dolutegravir and 10mg of Lamivudine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve

it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.225ml of Dolutegravir and 0.56ml of Lamivudine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:
Inject the Three replicate injections of individual concentrations (50%,100%,150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Dolutegravir and Lamivudine and calculate the individual recovery and mean recovery values[16].

Robustness:
The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

For preparation of Standard solution:
Accurately weigh and transfer 10 mg of Dolutegravir and 10mg of Lamivudine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15ml of the above Dolutegravir and 0.375ml of the Lamivudine stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Effect of Variation of Flow Conditions:
The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded[17].

Effect of Variation of Mobile Phase Organic Composition:
The sample was analyzed by variation of mobile phase i.e. Methanol: TEA Buffer: Acetonitrile was taken in the ratio and 40: 40:20, 60:10:30 instead (50:25:25), remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION:

Analytical Method Development:

Optimised Chromatographic Condition

Mobile phase : Methanol: TEA Buffer pH 4.5: Acetonitrile (50:25:25% v/v/v)
Column : Altima C18 (4.6mm×150mm, 5.0 µm)
Flow rate : 1.0 ml/min
Wavelength : 225 nm
Column temp : 40ºC
Injection Volume : 10 µl
Run time : 7 minutes

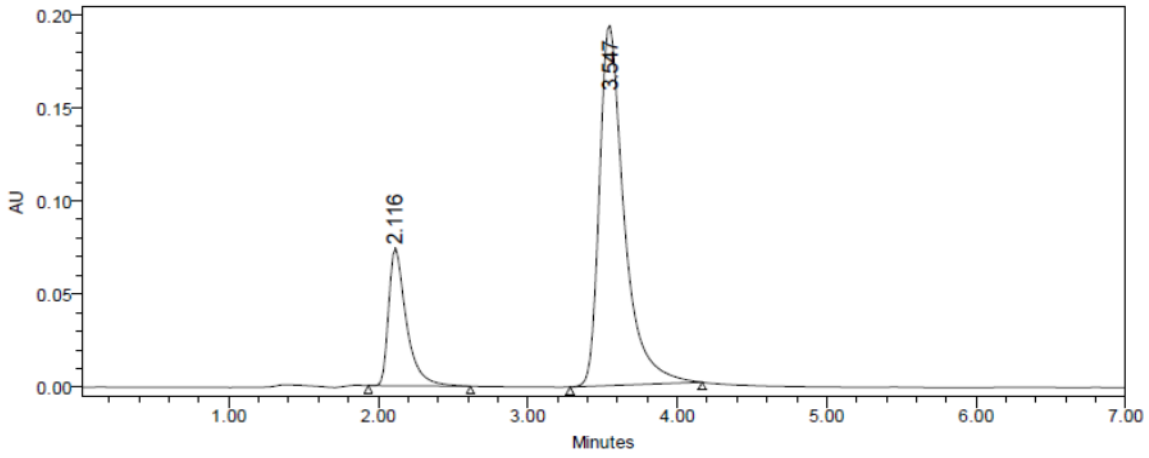

Fig-3: Optimized Chromatographic Condition

Analytical Method Validation:
The method was validated for linearity, accuracy, precision, specificity, LOD, and LOQ in accordance with ICH guidelines[18-21].

System Suitability: System suitability parameters were evaluated from retention times, tailing factor, capacity factor and theoretical plates of standard chromatograms (Table 3 & 4)[22].

Table-3: Results of System Suitability for Dolutegravir

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Dolutegravir	2.117	608452	71498	5643	1.9
2	Dolutegravir	2.118	606820	126412	5432	1.6
3	Dolutegravir	2.116	608452	126471	5123	1.6
4	Dolutegravir	2.109	595267	129859	5207	1.7
5	Dolutegravir	2.102	596608	124691	5481	1.6
Mean			**603119.8**			
Std. Dev			**6607.31**			
% RSD			**1.09**			

Table-4: Results of System Suitability for Dolutegravir

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Lamivudine	3.547	2234724	188631	5043	1.2	2.07
2	Lamivudine	3.539	2240080	2614821	5432	1.4	2.05
3	Lamivudine	3.547	2234724	2321451	5987	1.5	2.0
4	Lamivudine	3.565	2204466	2324710	5845	1.6	2.01
5	Lamivudine	3.537	2209574	2531247	5371	1.6	2.01
Mean			**2224714**				
Std. Dev			**16399.05**				
% RSD			**0.73**				

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantitates Dolutegravir and Lamivudine in drug product[23].

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Dolutegravir and Lamivudine in pharmaceutical dosage form was found to be 99.6%.

Linearity:

Different standard solutions were prepared by diluting standard stock solution with mobile phase in the concentration range 5-25 µg mL-1 for Dolutegravir and 12.5-50 µg mL-1 for Lamivudine respectively. Diluted samples were injected and chromatograms were taken under standard chromatographic conditions[24]. The peak area was plotted against corresponding concentrations to obtain the calibration graphs (Fig. 4 & 5).

Table-5: Linearity Data of Dolutegravir:

Concentration µg/ml	Average Peak Area
5	205035
10	381239
15	561128
20	740162
25	909922

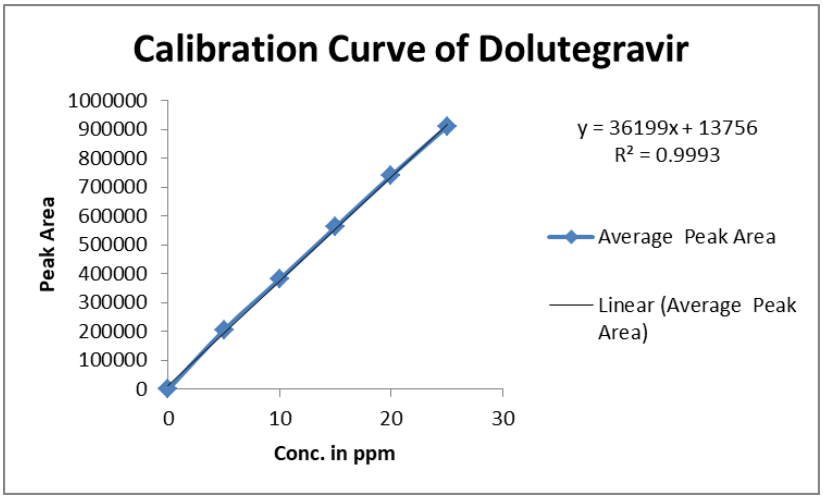

Fig-4: Calibration Graph for Dolutegravir

Linearity Plot: The plot of Concentration (x) versus the Average Peak Area (y) data of Dolutegravir is a straight line.

Y = mx + c
Slope (m) = 36199
Intercept (c) = 13756
Correlation Coefficient (r) = 0.999

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 13756. These values meet the validation criteria[25].

Table-6: Linearity Data of Lamivudine:

Concentration µg/ml	Average Peak Area
12.5	757881
12.5	757881
25	1458941
37.5	2132457
50	2901811

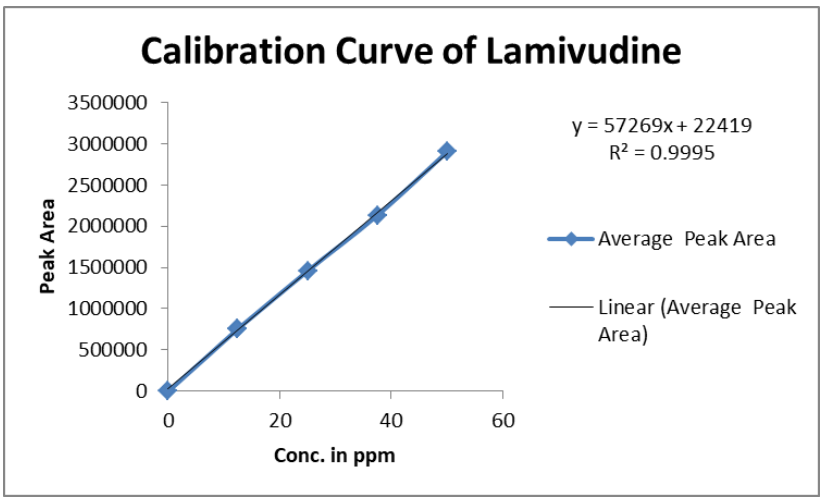

Fig-5: Calibration Graph for Lamivudine

Linearity Plot: The plot of Concentration (x) versus the Average Peak Area (y) data of Lamivudine is a straight line.

Y = mx + c
Slope (m) = 57269
Intercept (c) = 22419
Correlation Coefficient (r) = 0.999

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.
Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 22419. These values meet the validation criteria.

Precision

Precision of analytical method was expressed in relative standard deviation (RSD) of a series of measurements. The intra-day and inter-day precisions of the proposed methods were determined by estimating the corresponding responses (i.e. three concentrations/three replicates each) of the sample solution on the same day and on three different days, respectively (Table 7 & 8)[26].

Table-7: Results of Repeatability for Dolutegravir:

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Dolutegravir	2.108	602223	128898	2586	1.6
2	Dolutegravir	2.105	607748	129233	2947	1.4
3	Dolutegravir	2.113	607302	127409	2468	1.6
4	Dolutegravir	2.109	608674	127047	2146	1.9
5	Dolutegravir	2.109	607376	129859	2307	1.7
Mean			**606665**			
Std. Dev			**2542.3**			
% RSD			**0.42**			

Table-8: Results of Method Precision for Lamivudine:

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Lamivudine	3.552	2220333	2231111	1.6	2371
2	Lamivudine	3.550	2221573	2674210	1.6	2841
3	Lamivudine	3.564	2215483	2231261	1.5	2816
4	Lamivudine	3.564	2217379	2421301	1.5	2872
5	Lamivudine	3.565	2211255	2324710	1.6	2845
Mean			**2217205**		**1.6**	**2841**
Std. Dev			**4100.8**			
% RSD			**0.18**			

Intermediate Precision:

Day 1:

Table-9: Results of Intermediate Precision for Dolutegravir

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Dolutegravir	2.108	596608	128898	2547	1.6
2	Dolutegravir	2.105	598959	129233	2944	1.4
3	Dolutegravir	2.113	595728	127409	2361	1.6
4	Dolutegravir	2.109	594485	127047	2546	1.9
5	Dolutegravir	2.109	595267	129859	2207	1.7
6	Dolutegravir	2.102	596608	124691	2481	1.6
Mean			**596209**			
Std. Dev			**1718.7**			
% RSD			**0.29**			

Table-10: Results of Intermediate Precision for Lamivudine

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Lamivudine	3.552	2207732	2231134	8371	1.5	2.04
2	Lamivudine	3.550	2202266	2674210	6841	1.6	2.03
3	Lamivudine	3.564	2209375	2247461	7816	1.6	2.01
4	Lamivudine	3.564	2204037	2454301	8872	1.6	2.05
5	Lamivudine	3.565	2204466	2324710	4845	1.6	2.02

6	Lamivudine	3.537	2209574	2531247	8371	1.6	2.03
Mean			**2205575**				
Std. Dev			**2899.8**				
% RSD			**0.13**				

Day 2:

Table-11: Results of Intermediate Precision Day 2 for Dolutegravir

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Dolutegravir	2.102	602155	127998	5586	1.5
2	Dolutegravir	2.105	603662	134844	5636	1.6
3	Dolutegravir	2.112	603931	161103	5432	1.6
4	Dolutegravir	2.113	607302	127409	5468	1.6
5	Dolutegravir	2.109	608674	127047	5146	1.9
6	Dolutegravir	2.109	607376	129859	5307	1.7
Mean			**605516.7**			
Std. Dev			**2602.622**			
% RSD			**0.42**			

Table-12: Results of Intermediate Precision for Lamivudine

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Lamivudine	3.537	2241579	2263528	2371	1.6	7.98
2	Lamivudine	3.552	2236409	2224418	2414	1.6	6.4
3	Lamivudine	3.560	2239093	2233725	2384	1.6	8.9
4	Lamivudine	3.564	2215483	2231261	2816	1.5	8.3
5	Lamivudine	3.564	2217379	2421301	2872	1.5	7.5
6	Lamivudine	3.565	2211255	2324710	2845	1.6	5.3
Mean			**2226866**				
Std. Dev			**13567.02**				
% RSD			**0.60**				

Accuracy: Accuracy is the closeness of the test results obtained by the method to the true value. Recovery studies were carried out by addition of standard drug to the pre analysed sample at 3 different concentration levels (50, 100 and 150 %) taking into consideration percentage purity of added bulk drug samples. It was determined by calculating the recovery Dolutegravir and Lamivudine by standard addition method[27].

Table-13: The Accuracy Results for Dolutegravir

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	287774	7.5	7.56	100.8	99.6%
100%	551495	15	14.8	98.6	
150%	825175	22.5	22.4	99.5	

Table-14: The Accuracy Results for Lamivudine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1104782	18.75	18.73	100%	100%
100%	2105321	37.5	37.4	99.9%	
150%	3211306	56.25	56.21	100%	

Limit of Detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value[28].

LOD= 3.3 × σ / s

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

Dolutegravir:

=0.2µg/ml

Lamivudine:

=2.3µg/ml

Limit of Quantitation

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined[29].

LOQ=10×σ/S

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

Dolutegravir:

= 0.8µg/ml

Lamivudine:

= 7.04µg/ml

Robustness

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Dolutegravir and Lamivudine. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Dolutegravir and Lamivudine were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count[30-32].

Table-15: Results for Robustness of Dolutegravir

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	607323	2.102	5586	1.7
Less Flow rate of 0.9 mL/min	674735	2.330	5231	1.7
More Flow rate of 1.1 mL/min	1408920	1.950	5234	1.7
Less organic phase	606093	2.290	5643	1.4
More organic phase	603559	1.998	5298	1.5

Table-16: Results for Robustness of Lamivudine

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	558777	3.537	5371	1.6
Less Flow rate of 0.9 mL/min	2505636	3.885	5324	1.7
More Flow rate of 1.1 mL/min	1408920	3.263	5098	1.7
Less organic phase	2239255	4.435	5239	1.2
More organic phase	2300346	3.009	5647	1.0

SUMMARY AND CONCLUSION:

A RP-HPLC method is developed and validated as per ICH guidelines for simultaneous estimation of Dolutegravir and Lamivudine in bulk form and marketed pharmaceutical dosage forms.

In present study an attempt has been made to modify experimental condition, in order to estimate simultaneously the Dolutegravir and Lamivudine in combination. The mobile phase was selected after trying various combinations of polar solvents. The proportion of solvents and variation of buffers was found to be quite critical as slight variation in it adversely affected the resolution of peaks. Considering all the fact the following parameter were finally fixed for this method:

Equipment : High performance liquid chromatography equipped with WATERS, software: Empower 2, Auto Sampler and 996 PDA detector
Column : Altima C18 (4.6×150mm, 5.0 µm)
Mobile phase : Methanol: TEA Buffer pH 4.5: Acetonitrile (50:25:25)
Mode : Isocratic
Flow rate : 1.0 mL per min
Wavelength : 225 nm
Injection volume : 10 µl
Column oven : 40^0C
Run time : 7.0min

The proposed method was found to be rapid, accurate, precise, specific, robust and economical. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. This method is also having an advantage than reported method that the retention time of both the drugs is below 5 mins and both the drugs can be assayed with the short time. Thus the method is not time consuming and can be used in laboratories for the routine analysis of combination drugs.

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